Analysis of organic acids in selected forest litters of Northeast China

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Abstract: Larch (*Larix olgensis*), Manchurian ash (*Fraxinus mandshurica*), Korean pine (*Pinus koraiensis*) and White birch (*Betula platyphylla*) are the major planting species in northeast China. The samples of forest litters were collected from the stands of the above 4 species in Laoyeling and Jianlagou experiment stations of Maorshan Exp. Forest Farm (45º12′-45º30′N, 127º30′-127º48′E), Northeast Forestry University, in early October 2002. Quantitative analysis and qualitative analysis were carried out on the organic acids existing in freshly fallen litters (L layer) and hemi-decomposed litters (F layer) of the four forest species by using Gas Chromatogram system. A wide variety of organic acids were identified, including oxalic, malonic, fumaric, succinic, maleic, malic, citric, C_{16}:0, C_{18}:0, C_{18}:1, C_{18}:2, C_{18}:3 and C_{20}:0 acids. In respect of L litters of all samples, the oxalic acid content (over 30 mg/g) was the highest of the seven low-molecular-weight organic acids identified, while the content of oleic or linoleic (above 40 mg/g) was found to be highest among the six high aliphatic acids identified. As to F litters, oxalic acid content was also the highest, followed by linoleic and oleic. For the same tree species or the same forest, the kinds and contents of organic acids in L litters were more abundant than that in F litters.

Key words: Forest litter; Organic acid; Capillary gas chromatography
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Introduction

Litter fall is one of the major global carbon fluxes. Annual litter fall was estimated to be 6.53 Pg dm·a^{-1} (1Pg=10^{15} g, dry matter) in Eurasian forests (Liu *et al.* 2003). Organic acids in forest litters play important role in forests and forest soils. Nowadays, more attentions have been being paid to the importance of forest litters and some researchers conducted studies in this very aspect (Fox *et al*.1990; McColl 1997). Organic acids can form stable complexes with metals and therefore can affect metal solubility and phosphorous availability (Pohlman 1988; Fox *et al*.1992; Lu 1998). The specific organic acids appeared and their concentrations determine the degree to which many soil processes are affected (Shen 1997).

Although the organic acids with high concentrations have been found in forest soils supporting a variety of forest ecosystems (Fox *et al.*1990), there is little information in the literature on kinds and amounts of organic acids in forest litters (Pohlman 1988). Since organic acids have large impacts on forest soil and ecosystem processes, knowledge of suite of organic acids present in litters above the soils would be valuable. The purpose of this work was to characterize the organic acids present in a group of forest litters of northeast China.

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Materials and methods

Collections of litter samples

Samples of forest litters were collected from Laoyeling and Jianlagou experiment stations of Maorshan Exp. Forest Farm (45º12′-45º30′N, 127º30′-127º48′E), Northeast Forestry University in early October 2002. Maorshan Mountain is about 300 m in elevation on the west side of Zhangguangcai Range of Changbai Mountain and is in southeast of Harbin, Heilongjiang Province. In this area the vegetation type are natural secondary broad-leaved forests and/or mono-species plantations and zonal soil is Dark Brown Forest Soil from granitic parent material.

The new-fallen litters (L-layer) and more decomposed litters (F-layer) of Larch (*Larix olgensis*), Manchurian ash (*Fraxinus mandshurica*), Korean pine (*Pinus koraiensis*) and White birch (*Betula platyphylla*) stands from 13 plots were collected respectively. Random samples from L and F layers were collected at each plot and placed in polyethylene-lined paper bags. Samples were kept on ice during transportation to the laboratory. The L litters were hand-sorted to exclude coarse branches and litter of other species. All samples were air-dried, ground in an electric grinder and stored in plastic barrels prior to use. Sites and circumstances of collections were listed in Table 1.

Material treatment –lixiviation and esterification

We weighed out 0.500-g sample of ground litter materials, placed it in 50-mL clean and dried polyethylene bottle and added 10-mL of 7:100(v:v) H_2SO_4:CH_3OH solution to the bottle. The bottle was capped and heated in 60°C water bath where organic acids were lixiviated and esterification

took place. Samples were removed after 24 h and filtered through buchner funnel, and the bottle was cleaned with methanol (CH_3OH) three times. The filtrates were totally removed into a separatory funnel containing 20-mL deionized water and the esters of different organic acids were extracted with chloroform (CH_3Cl) (5-mL, 3 times). After separation, extraction in underlying layer was dewatered through sodium sulfate anhydrous (Na_2SO_4) column (About 5-mm sodium sulfate anhydrous in thick was placed in a glass column and fixed with fiberglass; sodium sulfate anhydrous and fiberglass were dried at 250 °C for 4 h). The filtrates dried were collected with test tube, and 1-µL filtrate was injected into the Capillary Gas Chromatography (GC) system and analyzed.

Table 1. Sites and collections of forest litter samples

Exp. station	Site No.	Forest condition	Status of collecting samples
Laoyeling	Lao Larch I	20-yr-old Larch plantation, gentle slope, low slope position, thick Dark Brown Forest Soil	L litter, F litter
	Lao Larch II	20-yr-old Larch plantation, gentle slope, mid slope position, thick Dark Brown Forest Soil	L litter, F litter
	Lao Ash I	20-yr-old Ash plantation, gentle slope, thick Dark Brown Forest Soil	L litter, F litter
	Lao Pine Birch	35-yr-old Korean pine plantation, Birch naturally invaded, 14° west slope, mid slope position, moderate Dark Brown Forest Soil	L litter (separated Pine and Birch), F litter (not separated Pine and Birch)
Jianlagou	Jian Larch I	12-yr-old Larch plantation, gentle slope, low slope position, moderate Dark Brown Forest Soil	L litter, F litter
	Jian Larch II	20-yr-old Larch plantation, gentle slope, low slope position, thick Dark Brown Forest Soil	L litter, F litter
	Jian Larch III	38-yr-old Larch plantation, flat slope, moderate Dark Brown Forest Soil	L litter, F litter
	Jian Ash I	12-yr-old Ash plantation, gentle slope, low slope position, moderate Dark Brown Forest Soil	L litter, F litter
	Jian Ash II	20-yr-old Ash plantation, gentle slope, low slope position, moderate Dark Brown Forest Soil	L litter, F litter
	Jian AshIII	12-yr-old Ash plantation, flat slope, thick Dark Forest Brown Soil	L litter, F litter
	Jian Birch I	Natural secondary broad-leaved forest dominated by 30 to 40-yr-old Birch, flat slope, moderate Dark Brown Forest Soil	L litter, F litter
	Jian Birch II	Middle-aged Birch forest, gentle slope, mid slope position, thick Dark Brown Forest Soil	L litter, F litter
	Jian Pine Birch	About 30-yr-old Korean pine and Birch mixed forests, flat slope, moderate Dark Brown Forest Soil	L litter (separated Pine and Birch), F litter (not separated Pine and Birch)

Instrument and analytical conditions

Analytical instrument: Agilent 6890-N Gas Chromatography (made in Agilent Company USA)

Chromatogram column: 30m×0.25mm×0.53µm FFAP organic-acid column, at a flow rate of 2.5 $mL \cdot min^{-1}$

Injector temperature: 230 °C

Carrier gas: nitrogen (N_2), purity≥99.999%

Program temperature: 120 °C (holding 2 min) $\xrightarrow{10℃/min}$ 160℃ $\xrightarrow{20℃/min}$ 200 °C (holding 20 min)

Injection volume: 1µL, Spill ratio: 20:1

Detector: FID, temperature: 250°C

Identification and quantification

Organic acids were identified by comparing retention times (Rt) of unknowns with those of reagent-grade organic acid standards at the same chromatogram conditions. Quantitative analysis was operated according to exterior-standard method, and contents of different organic acids were calculated with peak areas. Standard reagents of oxalic, malonic, fumaric, succinic, maleic, malic, citric, C_{16}:0, C_{18}:0, C_{18}:1, C_{18}:2, C_{18}:3 and C_{20}:0 acids were all provided by Sigma Company USA.

Results and analysis

Organic acid composition

The typical GC chromatograms of organic acids from L litters of natural birch (Jian Birch I), planted larch (Lao Larch II), birch-mixed pine (Jian Pine Birch), pine-mixed birch (Jian Pine Birch) and planted ash(Lao Ash I) forests were shown in Fig 1-5, respectively.

From Fig 1-5, it was found that organic acids were nearly ubiquitous in forest litters. A wide variety of organic acids have been identified including oxalic, malonic, fumaric, succinic, maleic, malic, citric, C_{16}:0, C_{18}:0, C_{18}:1, C_{18}:2, C_{18}:3 and C_{20}:0 acids. The suite of organic acids present in the forest litters was similar among the 13 sites, however, the kinds and relative abundance of organic acids varied with litter varieties. A number of chromatography peaks in filtrate still remained unidentified.

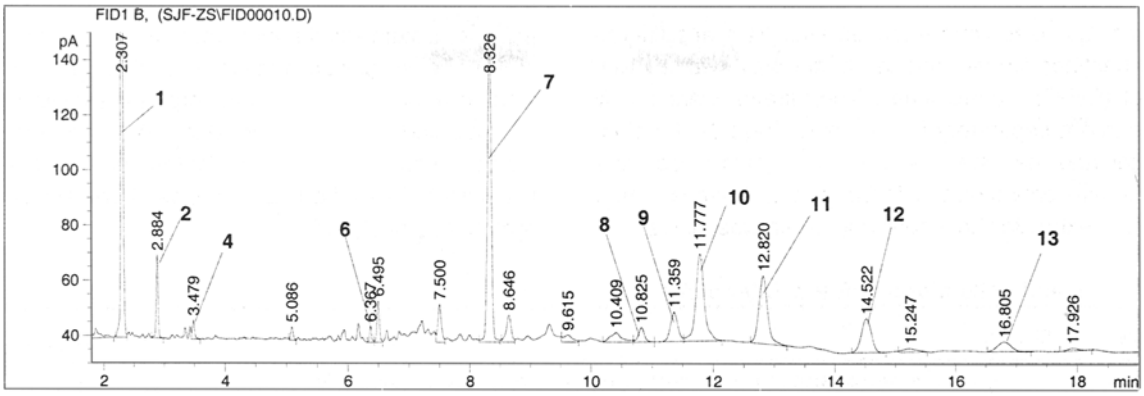

Fig. 1 The typical chromatograms of organic acids in litter sample of natural birch

Note: Fig.1-5: 1. oxalic; 2. malonic; 3. fumaric; 4. succinic; 5. maleic; 6. malic; 7. C_{16}:0; 8. citric; 9. C_{18}:0; 10. C_{18}:1; 11. C_{18}:2; 12. C_{18}:3;13. C_{20}:0

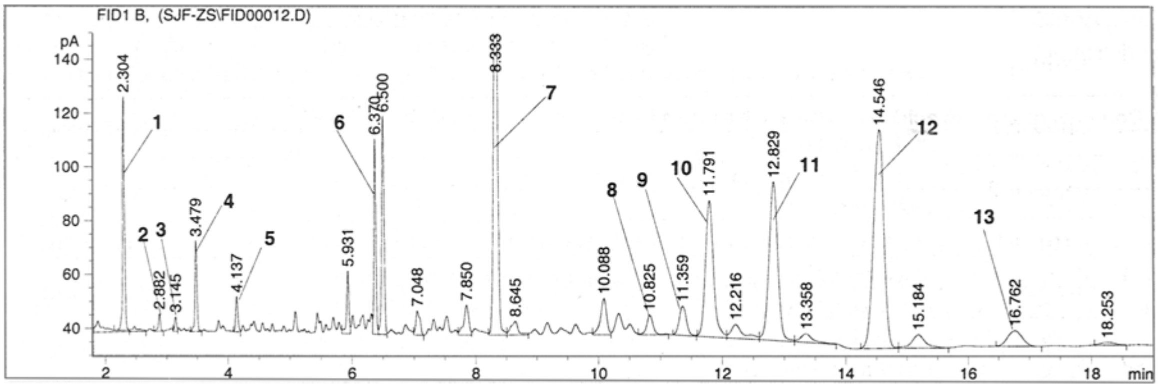

Fig. 2 The typical chromatograms of organic acids in litter sample of planted larch

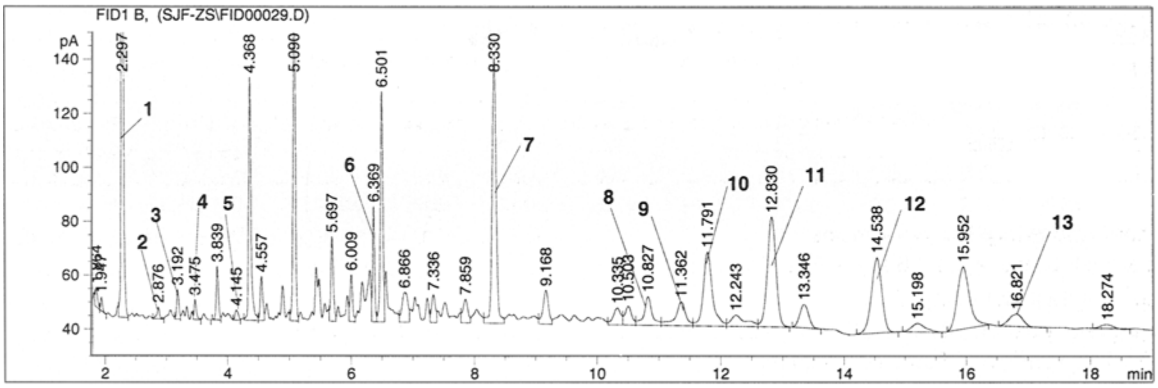

Fig. 3 The typical chromatograms of organic acids in litter sample of birch-mixed pine

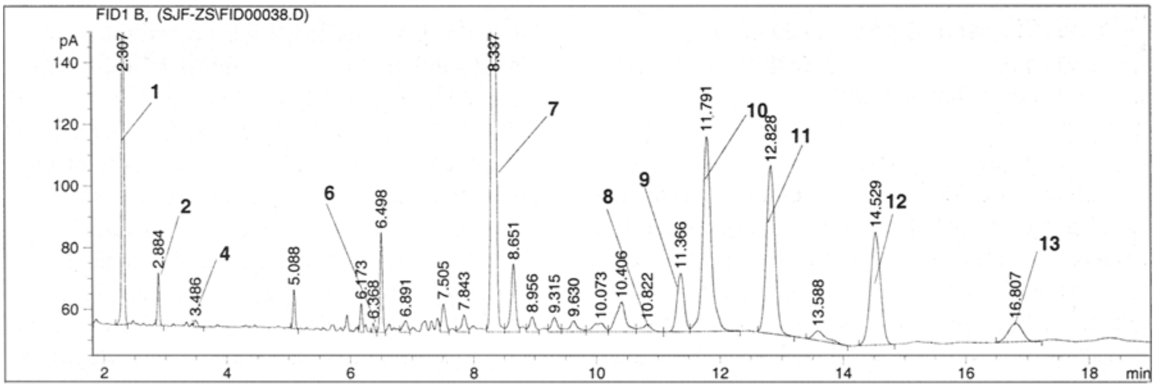

Fig. 4 The typical chromatograms of organic acids in litter sample of pine-mixed birch

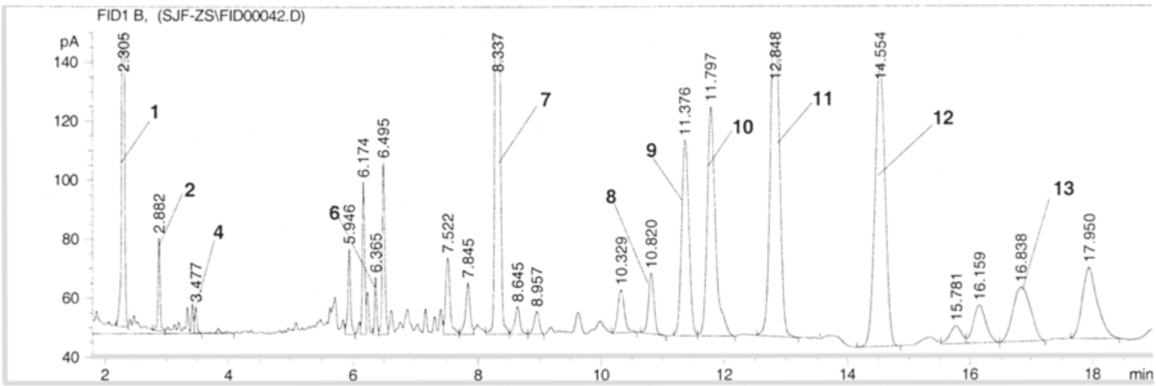

Fig. 5 The typical chromatograms of organic acids in litter sample of planted ash

Individual contents and variation

To gain a greater insight into the chemical and functional characteristics of the organic acids, filtrates from L and F litters were analyzed for organic acids content. Contents of different organic acids were shown in Table 2.

Table 2. Contents of organic acids in forest litter samples (mg/g)

Sample	Oxalic	Malonic	Fumaric	Succinic	Maleic	Malic	Citric	C_{16}:0	C_{18}:0	C_{18}:1	C_{18}:2	C_{18}:3	C_{20}:0
Ash L	34.71	3.17	0.12	0.46		4.44	6.87	5.80	2.60	35.03	59.34	5.58	1.80
n=4	±23.14	±0.34	±0.10	±0.14	—	±2.08	±5.59	±1.10	±1.87	±24.84	±44.97	±0.42	±1.44
Σ 159.92	(21.70)	(1.98)	(0.08)	(0.29)		(2.78)	(4.30)	(3.63)	(1.63)	(21.90)	(37.11)	(3.49)	(1.13)
Ash F	13.23	0.30	0.03	0.27		0.61	0.19	1.28	0.42	9.64	9.63	0.32	0.32
n=4	±2.93	±0.01	±0.01	±0.20	—	±0.42	±0.22	±0.42	±0.17	±4.59	±3.29	±0.24	±0.17
Σ 36.25	(36.48)	(0.84)	(0.08)	(0.75)		(1.69)	(0.52)	(3.52)	(1.17)	(26.60)	(26.57)	(0.89)	(0.88)
Larch L	14.13	0.54	0.89	2.20	0.74	10.35	3.00	5.34	0.60	28.46	41.16	7.76	0.73
n=5	±3.11	±0.15	±0.00	±0.48	±0.46	±2.83	±1.32	±3.00	±0.35	±15.31	±22.18	±4.74	±0.41
Σ 115.10	(12.27)	(0.47)	(0.08)	(1.92)	(0.64)	(8.99)	(2.60)	(4.64)	(0.52)	(24.73)	(35.76)	(6.74)	(0.64)
Larch F	6.91	0.20	0.02	0.55		1.00	0.09	0.76	0.16	5.23	5.45	0.14	
n=5	±3.15	±0.09	±0.00	±0.37	—	±0.32	±0.13	±0.16	±0.04	±1.01	±0.91	±0.02	—
Σ 20.50	(33.70)	(1.00)	(0.09)	(2.67)		(4.87)	(0.45)	(3.69)	(0.79)	(25.52)	(26.56)	(0.67)	
Birch L	63.48	3.54	0.11	0.45		2.08	1.53	8.48	1.37	44.67	42.90	2.83	0.95
n=4	±6.84	±0.75	±0.01	±0.06	—	±1.35	±0.13	±5.58	±0.74	±21.31	±26.34	±1.95	±0.09
Σ 172.40	(36.82)	(2.05)	(0.07)	(0.26)		(1.21)	(0.89)	(4.92)	(0.80)	(25.91)	(24.88)	(1.64)	(0.55)
Birch F	18.07	0.50	0.08	0.34		0.45	0.33	1.51	0.29	8.82	10.47	0.28	0.34
n=2	±5.21	±0.12	±0.00	±0.06	—	±0.09	±0.47	±0.91	±0.16	±5.65	±7.24	±0.02	±0.14
Σ 41.49	(43.56)	(1.14)	(0.19)	(0.79)		(1.03)	(0.76)	(3.47)	(0.67)	(21.26)	(25.24)	(0.65)	(0.77)
Pine L	39.57	0.37	0.52	0.50	0.30	5.94	2.78	2.90	0.43	16.74	28.33	2.79	0.55
n=2	±13.08	±0.19	±0.20	±0.12	±0.09	±3.10	±0.59	±1.59	±0.20	±8.34	±15.04	±0.94	±0.18
Σ 101.72	(38.90)	(0.37)	(0.51)	(0.49)	(0.30)	(5.84)	(2.73)	(2.85)	(0.42)	(16.45)	(27.86)	(2.74)	(0.54)
Mixed Birch and Pine F	15.77	0.38	0.02	0.20		0.57		1.15	0.22	7.88	8.55	0.09	0.34
n=2	±8.13	±0.17	±0.00	±0.00	—	±0.33	—	±0.08	±0.00	±0.03	±1.78	±0.00	±0.01
Σ 35.17	(44.84)	(1.09)	(0.06)	(0.57)		(1.61)		(3.26)	(0.64)	(22.40)	(24.30)	(0.25)	(0.97)

Notes: Figure in parenthesis is percentage of different organic acids in total acids identified;

Σ means total amount of organic acids in the sample ; — means not identified.

The total amounts of detected organic acids reached 115.10, 159.93, 172.40 and 101.72 mg·g^{-1} in the L litter samples of larch, ash, birch and pine respectively. Oxalic, malonic, fumaric, succinic, malic, citric, C_{16}:0, C_{18}:0, C_{18}:1, C_{18}:2,C_{18}:3 and C_{20}:0 acids were present in all L litters, whereas maleic was detected only in confer litters and was not found in broad-leafed materials. Oxalic, C_{18}:1 and C_{18}:2 were present at relatively high contents, generally above 30 mg/g and individually made up above 20% of the total. Contents of malic, citric, C_{16}:0, and C_{18}:3 were approximately in range of 2 to 10 mg/g and made up 1% to 9% of the total. The amounts of the other organic acids in L litter

samples generally sequenced as malonic>C_{20}:0, C_{18}:0> succinic>fumaric, of which the relative individual contents ranged from 0.5% to 2%.

As to F litter samples, the total amounts of organic acids were 20.50, 36.25, 41.49, 35.17 $mg \cdot g^{-1}$ in larch, ash, birch, and birch–pine–mixed forest respectively. Oxalic was still present in greater amounts than any other organic acids in most of the F litters, with contents ranging from 6.91 to 18.07 $mg \cdot g^{-1}$ and making up 33.70% to 44.84% of the detected acid content. Relative high content of linoleic (C_{18}:2) and oleic were also found in F litters. Contents of C_{16}:0 in F litters were above 1 $mg \cdot g^{-1}$. The quantities of malonic, succinic, malic, C_{18}:0 and C_{18}:3 ranged from 0.1 to 1 $mg \cdot g^{-1}$ in F litters and made up less than 1% individually. Citric was not found in pine–birch–mixed and was 0.09 to 0.33 $mg \cdot g^{-1}$ in other samples. C_{20}:0 was not detected in larch F litter, in other F litters its content was about 0.3 mg/g. Much lower quantities of fumaric were generally found in more decomposed F litters and were in range of 0.02-0.08 mg/g. Maleic was not detected in all F litters.

There existed significant differences between L and F litters of the same forest species in the composition and quantity of organic acids. Certain organic acids presented in L litters might be absent in the F litters beneath because of decomposition (Table 2). Amounts of organic acids in L litters were obviously higher than those in F litters. Oxalic, C_{18}:1 and C_{18}:2 were present in relatively higher contents, therefore their decrease in F litters were more evident than other acids.

Conclusions and discussion

Although a number of organic acids in litters still remain unidentified, we conclude that organic acids generally exist in forest litters of northeast China, with oxalic to be the dominant low-molecular-weight organic acid and oleic or linoleic as the main high aliphatic acids. The most abundant organic acids were generally present in freshly fallen litter of a given species.

Some early authors had found linoleic made a larger part of organic acids in larch or ash debris (Wang *et al.* 1997; Wu *et al.* 2000), but they had not detected low-molecular-weight organic acids, such as oxalic, malonic, fumaric, succinic, malic etc. Oxalic was found to be the most abundant low-molecular-weight organic acids in the litter leachates of incense cedar (*Calocedrus decurrens*), ponderosa pine (*Pinus ponderosa*), Douglas-fir (*Pseudotsuga menziesii*), longleaf pine (*Pinus palustris*), turkey oak (*Quercus laevis*), slash pine (*Pinus taeda*) and lyonia (*Lyonia spp.*) (Pohlman 1988; Fox 1990), which is consistent with the result of our study. It was further found that the amount of organic acids determined by Pohlman *et al* (1988) and Fox *et al* (1990) were substantially lower than that quantified in this paper. The most probable reason might be that of solvents, because the former leached samples with demineralized water (DMW) or diluted $NaOH/HNO_3$ solutions to obtain free-organic acids, whereas CH_3OH was used in this study to lixiviate total organic acids (free & combined). In addition, high aliphatic acids were not dealt with in Pohlman's (1988) and Fox's (1990) studies.

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